

## ABSTRACT

Methods for detecting G-protein coupled receptor (GPCR) activity; methods for assaying GPCR activity; and methods for screening for GPCR ligands, G-protein-coupled receptor kinase (GRK) activity, and compounds that interact 5 with components of the GPCR regulatory process are described. Included are methods for expanding ICAST technologies for assaying GPCR activity with applications for ligand fishing, and agonist or antagonist screening. These methods include: engineering seronine/threonine phosphorylation sites into known or orphan GPCR open reading frames in order to increase the affinity of arrestin for 10 the activated form of the GPCR or to increase the reside time of arrestin on the activated GPCR; engineering mutant arrestin proteins that bind to activated GPCRs in the absence of G-protein coupled receptor kinases which may be limiting; and engineering mutant super arrestin proteins that have an increased affinity for activated GPCRs with or without phosphorylation. These methods are intended to 15 increase the robustness of the GPCR/ICAST technology in situations in which G-protein coupled receptor kinases are absent or limiting, or in which the GPCR is not efficiently down-regulated or is rapidly resensitized (thus having a labile interaction with arrestin). Included are also more specific methods for using ICAST complementary enzyme fragments to monitor GPCR homo- and hetero- 20 dimerization with applications for drug lead discovery and ligand and function discovery for orphan GPCRs.